

MARKED SET OF AMENDED CLAIMS

60. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template, [The method of claim 44 wherein] and no bumper primers are used in the strand displacement amplification,

wherein steps (a), (b), and (c) occur at the same time.

78. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template, and the strand displacement reaction [The method of claim 45 wherein the strand displacement amplification in step (c)] utilizes an unequal effective concentration ratio of the first SDA primer to the second SDA primer, wherein the first and second SDA primers form a set of primers, and wherein unequal populations of complementary first and second amplified strands of the target nucleic acids are formed, [ in step (c)]

wherein steps (a), (b), and (c) occur at the same time.

100. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

wherein steps (a), (b), and (c) occur at the same time [The method of claim 44 wherein] and at least one of the first and second SDA primers is labeled.

106. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and  
(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

wherein steps (a), (b), and (c) occur at the same time,

[The method of claim 44]wherein allele specific strand displacement amplification is carried out for target nucleic acids of interest which encode a gene with two or more known alleles,

wherein the upstream and downstream ligation probe sequences are selected to be specific for a particular allele of the gene, so that the ligation probes contact, proximate the 3' end of the upstream probe or proximate the 5' end of the downstream probe, a portion of the target nucleic acid sequence which is determinative of the allele,

wherein, if the target nucleic acid does not contain the sequence determinative of the allele for which the ligation probes are selected, the juxtaposed terminus of one of the probes is misaligned, so that, in step (b), the ligation probes are ligated only if the target nucleic acid sequence contains the allele determinative sequence, and

wherein, in step (c), amplicons are produced by strand displacement amplification if the allele determinative sequence is contained within the target nucleic acid sequence.

111. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, wherein each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

wherein steps (a), (b), and (c) occur at the same time and [The method of claim 44 wherein] the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together, the method further comprising rendering capable of being ligated together the upstream and downstream oligonucleotide ligation probes prior to ligating the probes together in step (b).

117. (Once Amended) The [A] method of claim 116 wherein one or more terminal nucleotides are removed by an exonuclease.

133. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template, and [The method of claim 120 wherein] no bumper primers are used in the strand displacement amplification,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

136. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template, and [The method of claim 124 wherein] the first and second SDA primers are contained within a branched primer structure, having the capacity to support strand displacement amplification, [wherein] and the branched primer is attached to the capture site,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

160. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip [The method of claim 120] and [wherein] the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together, the method further comprising rendering capable of being ligated together the upstream and downstream oligonucleotide ligation probes prior to ligating the probes together in step (b).



166. (Once Amended) The [A] method of claim 165 wherein one or more terminal nucleotides are removed by an exonuclease.

176. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template, and [The method of claim 124 wherein] the strand displacement amplification [in step (c)] utilizes an unequal effective concentration ratio of the first SDA primer to the second SDA primer, wherein the first and second SDA primers form a set of primers, and wherein unequal populations of complementary first and second amplified strands of the target nucleic acids are formed, [in step (c)]

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

204. (Once Amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip,

[The method of claim 120]wherein allele specific strand displacement amplification is carried out for target nucleic acids of interest which encode a gene with two or more known alleles,

wherein the upstream and downstream ligation probe sequences are selected to be specific for a particular allele of the gene, so that the ligation probes contact, proximate the 3' end of the upstream probe or proximate the 5' end of the downstream probe, a portion of the target nucleic acid sequence which is determinative of the allele,

wherein, if the target nucleic acid does not contain the sequence determinative of the allele for which the ligation probes are selected, the juxtaposed terminus of one of the probes is misaligned, so that, in step (b), the ligation probes are ligated only if the target nucleic acid sequence contains the allele determinative sequence, and

wherein, in step (c), amplicons are produced by strand displacement amplification if the allele determinative sequence is contained within the target nucleic acid sequence.